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I, RONALD MAXWELL MAY, ASSISTANT DIRECTOR PATENT OPERATIONS, hereby certify that the annexed are true copies of the Provisional specification and drawing(s) as lodged on 19 October 1990 in connection with Application No. PK 2896 for a patent by BIOTA SCIENTIFIC MANAGEMENT PTY LTD lodged on 19 October 1990.

I further certify that pursuant to the provisions of section 50(1) of the Patents Act 1952 a complete specification was lodged on 24 April 1991 in respect of Applications PJ 9800, PK 2896, PK 4537 and has been allocated No. 75338/91.

I further certify that the annexed documents are not, as yet, open to public inspection.

WITNESS my hand this Seventeenth day of May 1991.

RONALD MAXWELL MAY
ASSISTANT DIRECTOR PATENT OPERATIONS

APPLICANT: BIOTA SCIENTIFIC MANAGEMENT PTY LTD

NUMBER:

FILING DATE:

COMMONWEALTH OF AUSTRALIA

The Patents Act 1952

PROVISIONAL SPECIFICATION FOR THE INVENTION ENTITLED:

"CHEMICAL COMPOUNDS"

This invention is described in the following statement:

CHEMICAL COMPOUNDS

This invention relates to a new class of chemical compounds and to their use in medicine. In particular the invention concerns new 4-substituted 2,3-didehydro derivatives of α-D-neuraminic acid, methods for their preparation, pharmaceutical formulations thereof and their use as antiviral agents.

Enzymes with the ability to cleave N-acetyl neuraminic acid (NANA), also known as sialic acid, from other sugars are present in many microorganisms. These include bacteria such as Vibrio cholerae, Clostridium perfringens, Streptococcus pneumoniae, and Arthrobacter sialophilus, and viruses such as influenza virus, parainfluenza virus, mumps virus, Newcastle disease virus, fowl plague virus, and Sendai virus. Most of these viruses are of the orthomyxovirus or paramyxovirus groups, and carry a neuraminidase activity on the surface of the virus particles.

Many of the neuraminidase-possessing organisms are major pathogens of man and/or animals, and some, such as influenza virus, Newcastle disease virus, and fowl plague virus, cause diseases of enormous economic importance.

It has long been thought that inhibitors of neuraminidase activity might prevent infection by neuraminidase-bearing viruses. Most of the known neuraminidase inhibitors are analogues of neuraminic acid, such as 2-deoxy-2,3-dehydro-N-acetylneuraminic acid (DANA) and its derivatives. See, e.g., Meindl et al., Virology 1974 58 457-63. The most active of these is 2-deoxy-2,3-dehydro-N-trifluoroacetyl-neuraminic acid (FANA), which inhibits multi-cycle replication of influenza and parainfluenza viruses in vitro. See Palese et al., Virology 1974 59 490-498.

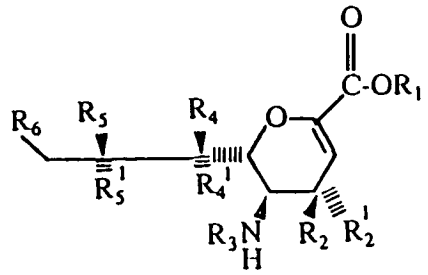
Table 1 below presents a listing of known 2,3-didehydro-N-acetylneuraminic acid derivatives. Many of these compounds are active against neuraminidase from v. cholerae or Newcastle disease virus as well as that from influenza virus. Neuraminidase in at least some strains of influenza or parainfluenza viruses is also inhibited by 3-aza-2,3,4-trideoxy-4-oxo-D-arabinoctonic acid δ-lactone and O-α-N-acetyl-D-neuraminosyl-)2--->3)-2-acetamido-2-deoxy-D-glucose Zakstel'skaya et al., Vop. Virol. 1972 17 223-28.

Neuraminidase from Arthrobacter sialophilus is inhibited by the glycols 2,3-dehydro-4-epi-N-acetyl-neuraminic acid, 2,3-dehydro-2-deoxy-N-acetylneuraminic acid and 5-acetamido-2,6-anhydro-2,3,5-trideoxy-D-manno-non-2-en-4-ulosonate, and by their methyl esters. See Kumar et al., Carbohydrate Res. 1981 94 123-130; Carbohydrate Res. 1982 103 281-285. The thio analogues 2-α-azido-6-thio-neuraminic acid and 2,3-dehydro-6-thioneuraminic acid, Mack & Brossmer, Tetrahedron Letters 1987 28 191-194, and the fluorinated analogue N-acetyl-2,3-difluoro-α-D-neuraminic acid, Nakajima et al., Agric. Biol. Chem. 1988 52 1209-1215, were reported to inhibit neuraminidase, although the type of neuraminidase was not identified. Schmid et al., Tetrahedron Letters 1958 29 3643-3646, described the synthesis of 2-deoxy-N-acetyl-α-D-neuraminic acid, but did not report its activity or otherwise against neuraminidase.

None of the known inhibitors of neuraminidase activity in vitro has been shown to posses antiviral activity in vivo, and indeed some, such as FANA, have specifically been shown to be inactive in vivo. Thus the conventional wisdon has accordingly considered that compounds exhibiting in vitro inhibition of viral neuraminidase would not effect an in vivo blockade of virus infection.

TABLE 1

Known 2,3-dehydro derivatives on N-acetylneuraminid acid



	R_1	R_2	R_2'	R_3	R_4	R_4'	R_5	R_5'	R_6
1	H	H	OH	CH_3CO-	H	OH	OH	H	OH
2	H	H	OH	NH_2CO-	H	OH	OH	H	OH
3	H	H	OH	HCO-	H	OH	OH	H	OH
4	H	H	OH	FCH_2CO-	H	OH	OH	H	OH
5	H	H	OH	F_2CHCO-	H	OH	OH	H	OH
6	H	H	OH	F_3CCO-	H	OH	OH	H	OH
7	H	H	OH	$ClCH_2CO-$	H	OH	OH	H	OH
8	H	H	OH	ICH_2CO-	H	OH	OH	H	OH
9	H	H	OH	$CNCH_2CO-$	H	OH	OH	H	OH
10	H	H	OH	NH_2CH_2CO-	H	OH	OH	H	OH
11	H	H	OH	$HSCH_2CO-$	H	OH	OH	H	OH
12	H	H	OH	$CH_2CONHCH_2CO-$	H	OH	OH	H	OH
13	H	H	OH	$(CH_3)_2NCH_2CO-$	H	OH	OH	H	OH
14	H	H	OH	$NH_2CH_2CH_2CO-$	H	OH	OH	H	OH
15	H	H	OH	$CH_3CONHCH_2CHCO-$	H	OH	OH	H	OH
16	H	H	OH	$HOOCCH_2CH_2CO-$	H	OH	OH	H	OH
17	H	H	OH	HOOCCH=CHCO-	H	OH	OH	H	OH
18	H	H	OH	$Neu5Acyl2enNHCOCH_2SCH_2CO-$	H	OH	OH	H	OH
19	H	H	OH	$HOCH_2CO-$	H	OH	OH	H	OH
20	H	H	OH	CH_3CH_2CO-	H	OH	OH	H	OH

	R_1	R_2	R_2	R_3	R_4	R_4	R_5	R_5	R_6
21	H	H	OH	$CH_3CH_2CH_2CO$-	H	OH	OH	H	OH
22	H	H	OH	C_6H_5CO-	H	OH	OH	H	OH
23	H	H	OH	$C_6H_5CH_2CO$-	H	OH	OH	H	OH
24	CH_3	H	OH	CH_3CO-	H	OH	OH	H	OH
25	CH_3	OH	H	CH_3CO-	H	OH	OH	H	OH
26	CH_3	H	OH	CH_3CO-	H	OH	OH	H	OTr
27	CH_3	OH	H	CH_3CO-	H	OH	OH	H	O(p-CH_3OC_6H
28	CH_3	H	OH	CH_3CO-	H	OH	OH	H	O(p-CH_3OC_6H
29	CH_3	=O		CH_3CO-	H	OH	OH	H	O(p-CH_3OC_6H
30	CH_3	=O		CH_3CO-	H	OH	OH	H	OTr
31	CH_3	=O		CH_3CO-	H	OH	OH	H	OH
32	H	=O		CH_3CO-	H	OH	OH	H	OH
33	H	H	OH	CH_3CO-	H	H	OH	H	OH
34	H	H	OH	CH_3CO-	H	OH	H	H	OH
35	H	H	OH	CH_3CO-	H	OH	OH	H	H
36	H	H	H	CH_3CO-	H	H	OH	H	OH
37	CH_3	H	CH_3COO-	CH_3CO-	H	H	CH_3COO-	H	CH_3COO-
38	CH_3	H	CH_3COO-	CH_3CO-	H	CH_3COO-	H	H	CH_3COO-
39	CH_3	H	CH_3COO-	CH_3CO-	H	CH_3COO-	CH_3COO-	H	H
40	CH_3	H	H	CH_3CO-	H	H	CH_3COO-	H	CH_3COO-
41	CH_3	H	$C_6H_5CH_2O$-	CH_3CO-	H	$C_6H_5CH_2O$-	$C_6H_5CH_2O$-	H	$C_6H_5CH_2O$-
42	CH_3	H	CH_3COO-	CH_3CO-	H	CH_3COO-	CH_3COO-	H	CH_3COO-
43	CH_3	H	CH_3COO-	CH_3CO	H	CH_3COO-	H	H	CH_3COO-
44	CH_3	H	CH_3COO-	CH_3CO	H	CH_3COO-	H	CH_3COO-	H
45	CH_3	H	CH_3COO-	CH_3CO	H	CH_3COO-	H	CH_3COO-	2αNeu5Ac
46	$C_6H_5CH_2$	H	CH_3COO-	CH_3CO	H	CH_3COO-	CH_3COO-	H	CH_3COO-

Compounds 1-18 P. Meindl, G. Bodo, P. Palese, J Schulman and H. Tuppy. Inhibition of Neuraminidase Activity by Derivatives c 2-Deoxy-2,3-dehydro-N-acetylneuraminic Acid. Virology 58, 457-463 (1974).

Compounds 19-23 P. Meindl and H. Tuppy. Ueber 2-Desoxy-2,3-deshydro-sialinsaeuren 1. Mitt. : Synthese und Eigenschaften von 2 Desoxy-2,3-deshydro-N-acylneuraminsaeuren and deren Methylestern. Mh. Chem. 100(4) 1295-1306 (1969)

Compounds 24-32 M. Flashner et al. Methyl-5-acetamido-2,6-anhydro-3,5-dideoxy-D-manno-non-2-en-4-ulosonate. Carbohydrat Research 103, 281-285 (1982).

Compounds 33-40 E. Zbiral et al. Synthesis of 2,7-, 2,8- and 2,9-Dideoxy and 2,4,7-Trideoxy-2,3-didehydro-N-acetylneuramini Acids and Their Behaviour Towards Sialidase from Vibrio cholerae Liebigs Ann Chem 1989, 159-165.

Compounds 41-42 T. Ogawa and Y. Ito. An Efficient Approach to Stereo-selective Glycosylation of N-Acetylneuraminic Acid : Us of Phenylselenyl Group as a Stereocontrolling Auxillary. Tetrahedron Letters 28, (49), 6221-6224 (1987).

Compounds 43-45 T. Goto et al. Synthesis of (α2-9) and (α2-8) Linked Neuraminylneuraminic Acid Derivatives. Tetrahedron Letter 27, (43), 5229-5232 (1986).

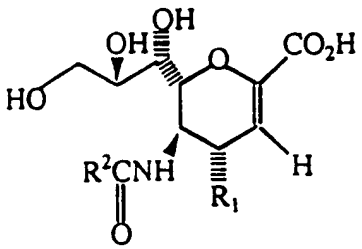
Compound 46 H. Ogura et al. Studies on Sialic Acids XV. Synthesis of α and β-O-Glycosides of 3-Deoxy-D-glycero-D-galacto-2 nonulopyranosonic Acid (KDN). Chem. Pharm. Bull. 36 (12), 4807-4813 (1988).

Meindl and Tuppy, Hoppe-Seyler's Z. Physiol Chem. 1969 350 1088, described hydrogenation of the olefinic double bond of 2-deoxy-2,3-dehydro-N-acetylneuraminic acid to produce the β-anomer of 2-deoxy-N-acetylneuraminic acid. This β-anomer did not inhibit Vibrio cholerae neuraminidase.

The most potent in vitro inhibitors of viral neuraminidase have thus been identified as compounds that are based on the neuraminic acid framework, and these are thought by some to be transition-state analogues. Miller et al., Biochem. Biophys. Res. Comm. 1978 83 1479. But while many of the aforementioned neuraminic acid analogues are competitive inhibitors of neuraminidases, to date, none has been reported as showing anti-viral activity in vivo. For example, although a half-planar, unsaturated 6-member ring system has been asserted to be important for inhibitory activity, see Dernick et al. in ANTIVIRAL CHEMOTHERAPY (K. K. Gauri ed.) Academic Press, 1981, at pages 327-336, some compounds characterized by such a system, notably FANA, have been reported not to possess in vivo anti-viral activity. See Palese and Schulman in CHEMOPROPHYLAXIS AND VIRUS INFECTION OF THE UPPER RESPIRATORY TRACT, Vol. 1 (J. S. Oxford ed.) CRC Press, 1977, at pages 189-205.

We have now found a novel class of 4-substituted 2,3-didehydro derivatives of α-D-neuraminic acid which are unexpectedly more active than their corresponding 4-hydroxy analogues and which are active in vivo.

The invention therefor provides in a first aspect compounds of formula (I)



(I)

wherein R^1 is $(alk)_xNR^3R^4$, CN or N_3

where alk is unsubstituted or substituted methylene,

x is 0 or 1

R^3 is hydrogen, C_{1-6}alkyl (e.g. methyl, ethyl), allyl,aryl (e.g. phenyl), aralykyl (e.g. phenC_{1-3}alkyl such as benzyl), amidine, NR^4R^5 or an unsaturated or saturated ring containing one or more heteroatoms (such as nitrogen, oxygen or sulphur),

R^4 is hydrogen or C_{1-6}alkyl (e.g. methyl, ethyl), allyl or NR^3R^4 forms an optionally substituted 5 or 6 membered ring optionally containing one or more additional heteroatoms (such as nitrogen, oxygen or sulphur), or R^3 and R^4 may be the same; and R^5 is hydrogen or C_{1-6}alkyl;

and

R^2 is hydrogen, substituted or unsubstituted C_{1-4}alkyl or aryl, and pharmaceutically acceptable salts of the compounds of formula (I) and their pharmaceutically acceptable derivatives thereof.

In the compounds of formula (I) the substituents (for example the group R^3 in the substituent R^1) may themselves bear substituents conventionally associated in the art of pharmaceutical chemistry with such substituents.

Preferably R^1 is NR^3R^4, in particular NH_2 or guanidino.

Preferably R^2 is methyl or halogen substituted methyl (e.g. FCH_2, F_2CH-, F_3C).

C_{1-4}alkyl as used herein includes both straight chain (e.g. methyl, ethyl) and branched chain (e.g. isopropyl, t-butyl) alkyl groups.

By pharmaceutically acceptable derivative is meant any pharmaceutically acceptable ester or salt of such ester of the compounds of formula (I) or any other compound which upon administration to the recipient is capable of providing (directly or indirectly) a compound of formula (I) or an antivirally active metabolite or residue thereof.

It will be appreciated by those skilled in the art that the compounds of formula (I) may be modified to provide pharmaceutically acceptable derivatives thereof at any of the functional groups in the compounds. Of particular interest as such derivatives are compounds modified at the C-1 carboxyl function or the C-7 or C-9 hydroxyl functions. Thus compounds of interest include C-1 alkyl (such as methyl or ethyl) or aryl (e.g. phenyl, benzoyl) esters of the compounds of formula (I), C-7 or C-9 esters of compounds of formula (I) such as acetyl esters thereof and C-7 or C-9 ethers such as phenyl ethers, benzyl ethers, p-tolyl ethers.

It will be appreciated by those skilled in the art that the pharmaceutically acceptable derivatives of the compounds of formula (I) may be derivatised at more than one position.

Pharmaceutically acceptable salts of the compounds of formula (I) include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene-p-sulphonic, tartaric, acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids. Other acids such as oxalic, while not in themselves pharmaceutically acceptable may be useful in the preparation of salts useful as intermediates in obtaining compounds of the invention and their pharmaceutically acceptable acid addition salts.

Salts derived from appropriate bases include alkali metal (e.g. sodium), alkaline earth metal (e.g. magnesium), ammonium and NR_4^+ (where R is C_{1-4}alkyl) salts.

References hereinafter to a compound of the invention includes the compounds of formula (I) and pharmaceutically acceptable salts and derivatives thereof.

The compounds of formula (I) possess antiviral activity. In particular these compounds are inhibitors of viral neuraminidase of orthomyxoviruses and paramyxoviruses, for exmaple the viral neuramindase of influenza A and B, parainfluenza, mumps, Sendai virus, Newcastle disease and fowl plague.

There is thus provided in a further aspect of the invention a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof for use as an active therapeutic agent in particular as an antiviral agent for example in the treatment of orthomyxovirus and paramyxovirus infections.

In a further or alternative aspect there is provided a method for the treatment of a viral infection, for example orthomyxovirus and paramyxovirus infections in a mammal including man comprising administration of an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof.

There is also provided in a further or alternative aspect use of a compound of the invention for the manufacture of a medicament for the treatment of a viral infection.

It will be appreciated by those skilled in the art that reference herein to treatment extends to prophylaxis as well as the treatment of established infections or symptoms.

It will be further appreciated that the amount of a compound of the invention required for use in treatment will vary not only with the particular compound selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will ultimately be at the discretion of the attendant physician or veterinarian. For administration by inhalation, the daily dosage as employed for treatment, according to the present invention, of an adult human of approximately 70 kg body weight will range from 1mg to 1000mg, preferably between 5mg and 500mg, and may take the form of single or multiple doses, e.g., one to six times a day. For oral administration, the daily dosage (again, for treatment of a 70kg adult) will typically range from about 1mg to 5g, preferably between 5mg and 2g, and may be given, for example, in single to four doses per day. It will therefore be convenient for a pharmaceutical composition of the present invention to contain active (antiviral) agent at a concentration in the range of 0.000001 to 100 mg/ml.

The desired dose may be presented in a single dose or as divided doses administered at appropriate intervals, for example as two, three, four or more sub-doses per day.

The compound is conveniently administered in unit dosage from for example containing 10 to 1500mg, conveniently 20 to 1000mg, most conveniently 50 to 700mg of active ingredient per unit dosage form.

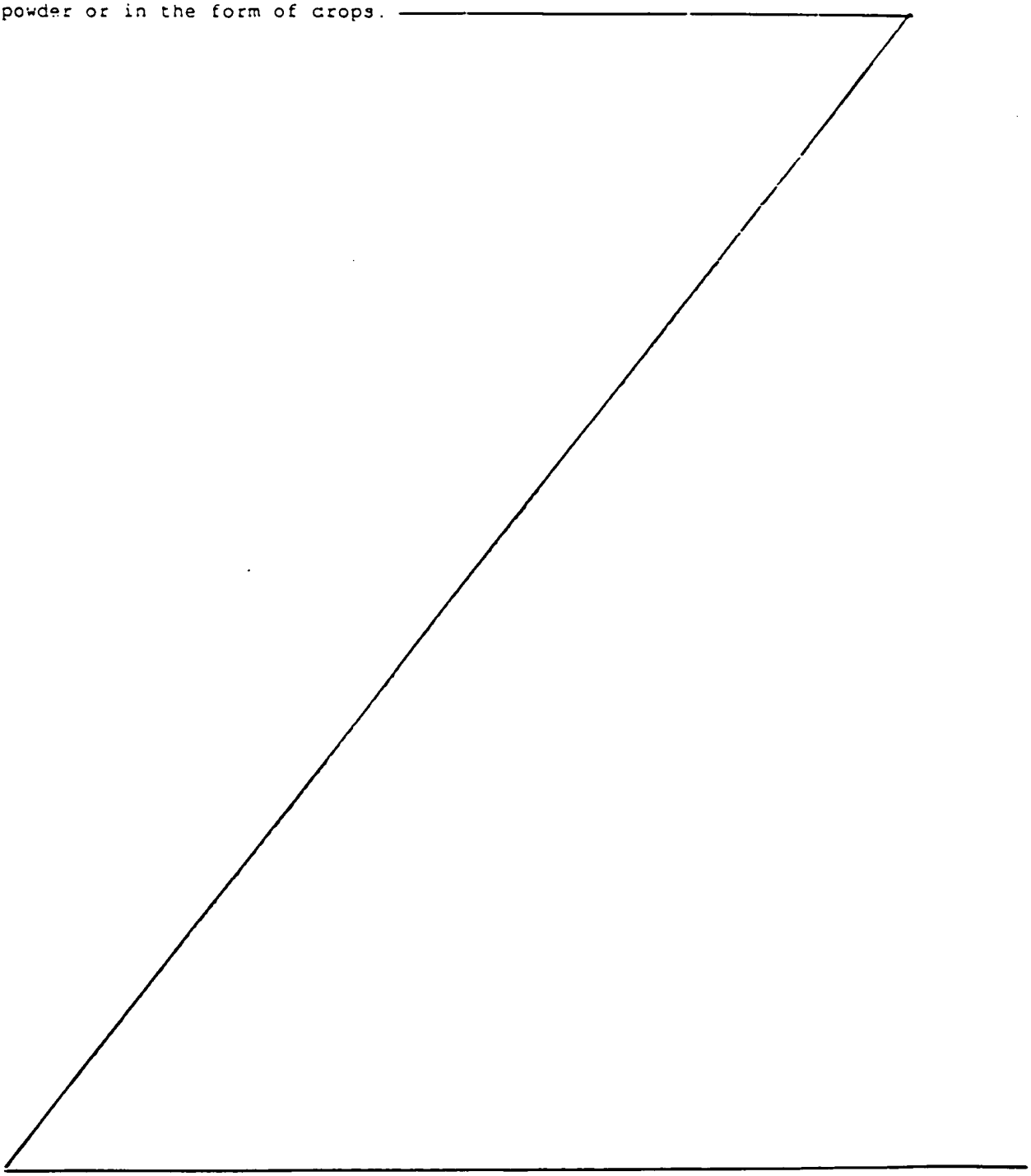
While it is possible that, for use in therapy, a compound of the invention may be administered as the raw chemical it is preferable to present the active ingredient as a pharmaceutical formulation.

The invention thus further provides a pharmaceutical formulation comprising a compound of the formula (I) or a pharmaceutically acceptable salt or derivative thereof together with a pharmaceutically acceptable carrier therefor.

The carrier must be 'acceptable' in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The pharmaceutical formulations may be in the form of conventional formulations for the intended mode of administration.

Preferably the formulation is suitable for intranasal administration which may be presented as a liquid spray or dispersible powder or in the form of drops.



Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents solubilising agents or suspending agents. Liquid sprays are conveniently delivered from pressurised packs which may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluroromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas.

When desired the formulations may be adapted to give sustained release of the active ingredient may be employed. The compounds of the invention may also be used in combination with other therapeutic agents, for example other anti-infective agents. In particular the compounds of the invention may be employed with other antiviral agents. The invention thus provides in a further aspect a combination comprising a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof together with another therapeutically active agent, in particular an antiviral agent.

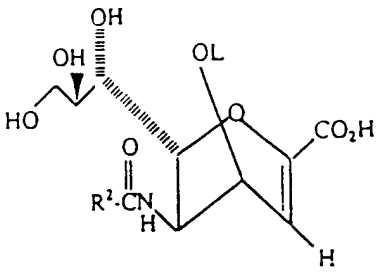
The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus such formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier therefor comprise a further aspect of the invention.

The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

When the compounds of the invention are used with a second therapeutic agent active against the same virus the dose of each compound may either be the same as or differ from that employed when each compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

The compounds of formula (I) and its pharmaceutically acceptable salts and derivatives may be prepared by any method known in the art for the preparation of compounds of analogous structure.

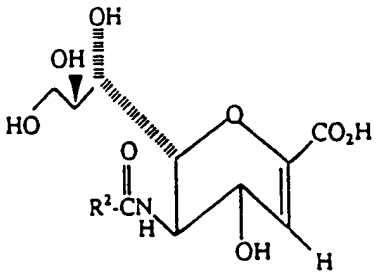
In one such process (A) a compound of formula (II)



(II)

wherein R^2 is as defined in formula (I), L is a leaving group (for example a sulphonic acid residue such as tosyl, mesyl, trifluoromesyl) or a protected derivative thereof with the appropriate nucleophile, for example nitrile.

The compounds of formula (II) may be obtained from the corresponding compounds of formula (III)



(III)

by inversion of the 4-OH group by methods known in the art for example by reaction with a Lewis acid (such as BF_3 etherate) followed by hydrolysis. The compounds of formula (III) are either known in the art or may be obtained by methods analogous to those for preparing the known compounds.

In a second method (B) the compounds of formula (I) may be prepared from other compounds of formula (I) by interconversion. Thus compounds of formula (I) wherein R^1 is NH_2 or CH_2NH_2 may be prepared by reduction of the corresponding azido or cyano analogues respectively.

Compounds wherein R^1 is NH alkyl or guanidino may be prepared by derivatisation of the corresponding compound wherein R^1 is NH_2.

As will be appreciated by those skilled in the art it may be necessary or desirable at any stage in the above described processes to protect one or more sensitive groups in the molecule to prevent

undesirable side reactions; the protecting group may be removed at any convenient subsequent stage in the reaction sequence.

The protecting groups used in the preparation of compounds of formula (I) may be used in conventional manner. See for example 'Protective Groups in Organic Chemistry' Ed. J. F. W. McOmie (Plenum Press 1973) or 'Protective Groups in Organic Synthesis' by Theodora W Greene (John Wiley and Sons 1981).

Conventional amino protecting groups may include for example aralkyl groups, such as benzyl, diphenylmethyl or triphenylmethyl groups; and acyl groups such as N-benzyloxycarbonyl or t-butoxycarbonyl. Thus, compounds of general formula (I) wherein one or both of the groups R_1 and R_2 represent hydrogen may be prepared by deprotection of a corresponding protected compound.

Hydroxy groups may be protected, for example, by aralkyl groups, such as benzyl, diphenylmethyl or triphenylmethyl groups, acyl groups, such as acetyl, silicon protecting groups, such as trimethylsilyl groups, or as tetrahydropyran derivatives.

Removal of any protecting groups present may be achieved by conventional procedures. Thus an aralkyl group such as benzyl, may be cleaved by hydrogenolysis in the presence of a catalyst (e.g. palladium on charcoal); an acyl group such as N-benzyloxycarbonyl may be removed by hydrolysis with, for example, hydrogen bromide in acetic acid or by reduction, for example by catalytic hydrogenation; silicon protecting groups may be removed, for example, by treatment with fluoride ion; tetrahydropyran groups may be cleaved by hydrolysis under acidic conditions.

Where it is desired to isolate a compound of the invention as a salt, for example as an acid addition salt, this may be achieved by treating the free base of general formula (I) with an appropriate acid, preferably with an equivalent amount, or with creatinine suiphate in a suitable solvent (e.g. aqueous ethanol).

The present invention is further described by the following examples which are for illustrative purposes only and should not be construed as a limitation of the invention.

Example 1 The preparation of Sodium 5-Acetamido-4-azido-2,3,4,5-tetradeoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate (4)(4-Azido-Neu5Ac2en)

Compounds are designated as in Figure 1.

Preparation of (2)

To an agitated solution of methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate (1) (1500 mg, 3.17 mmol) in a mixture of benzene (50 ml) and methanol (300 mg) was added dropwise $BF_3.Et_2O$ (12 ml) over thirty minutes under a nitrogen atmosphere at room temperature. The whole mixture was then allowed to stir at room temperature for 16 hours. The solution was diluted with ethyl acetate (250 ml), washed successively with saturated $NaHCO_3$ solution (30 ml x 3) and water (20 ml x 3), then evaporated to a small volume (about 10 ml), to which was added water (0.5 ml) and acetic acid (0. 5ml). The whole mixture was then stirred at room temperature for two days before being diluted with ethyl acetate (200 ml). The ethyl acetate solution was washed with 5% $NaHCO_3$ solution (30 ml x 2) and water (20 ml x 3), then evaporated to dryness. The residue was chromatographed (silica gel, ethyl

acetate as eluting solvent) to afford pure compound (2) (550 mg, 40%).

^{1}H-nmr ($CDCl_3$) δ (ppm); 1.95, 2.06, 2.08, 2.10, 2.35 (s, 15H, Acetyl CH_3 x 5), 3.80 (s, 3H, $COOCH_3$), 4.1-4.4 (m, 4H, H_4, H_5, H_6, H_9), 4.82 (dd, 1H, $J_{9,8}$ 1.8Hz, $J_{9,9}$, 12.3Hz, H_9), 5.27 (m, 1H, H_8), 5.45 (dd, 1H, $J_{7,8}$ 3.5Hz, H_7), 6.15 (d, 1H, $J_{3,4}$ 5.4Hz, H_3), 6.47(d, 1H, $J_{NH,5}$ 8.8Hz, -CONH).

Preparation of (3)

To a stirred solution of compound (2) (800 mg, 1.67 mmol) in anhydrous dichloromethane (10 ml) and dry pyridine (316 mg, 4 mmol) at -30 to -40° C, was added dropwise a solution of trifluoromethane sulphonic anhydride (Tf_2O) (556 mg, 2 mmole) in dichloromethane (2 ml) over 15 minutes.. The reaction mixture was then stirred at -30° C for 5 hours, and concentrated to dryness *in vacuo*. The residue was then dissolved in dry DMF (5 ml) containing a mixture of sodium azide (650 mg, 10 mmol) and tetrabutylammonium hydrogen sulphate (170 mg, 0.5 mmol). The reaction mixture was stirred at room temperature for 16 hours, and then evaporated to dryness under high vacuum. The residue was partitioned between ethyl acetate (200 ml) and water (50 ml). The organic layer was separated and washed with water (50 ml x 2), dried over Na_2SO_4, evaporated to leave a residue (780 mg), which was subjected to double chromatography (silica gel, the first solvent system was ethyl acetate/acetone: 8/1; the second solvent system was dichloromethane/water: 10/1) to afford a colourless

oil (3) (185 mg, 24%). MS. (FAB) 457 ($M^+ + 1$), 414($M^+ - N_3$). $[\alpha]^{20}_D$ + 19.1° (C1,MeOH). ir.($CHCl_3$))cm^{-1} 2100 ($-N_3$). 1748 (carbonyl). ^{1}H-nmr ($CDCl_3$) δ (ppm). 2.04, 2.05, 2.06, 2.12, (s, 12H, Acetyl CH_3 x 4). 3.79 (s, 3H, $COOCH_3$), 3.91 (ddd, 1H, $J_{5,NH}$ 8.4Hz, $J_{5,4}$ 8.8Hz, $J_{5,6}$9.9Hz, H_5), 4.17 (dd, 1H, $J_{9',8}$ 6.8 Hz, $J_{9',9}$, 12.5 Hz, $H_{9'}$), 4.42 (dd, 1H, $J_{4,3}$2.9Hz, $J_{4,5}$8.8Hz, H_4), 4.48 (dd, 1H, $J_{6,7}$2.3Hz, $J_{6,5}$ 9.9 Hz, H_6 4.46 (dd, 1H, $J_{9,8}$ 2.7 Hz, $J_{9,9'}$ 12.5 Hz, H_9), 5.31 (m, 1H, $J_{8,7}$ 5.2 Hz, $J_{8,9}$ 2.7 Hz, $J_{8,9'}$ 6.8 Hz, H_8), 5.45 (dd, 1H, $J_{7,6}$2.3Hz, $J_{7,8}$5.2Hz, H_7), 5.96 (d, 1H, $J_{3,4}$2.9Hz, H_3), 6.13 (d, 1H, $J_{NH,5}$8.4Hz, -CONH)

^{13}C-$_{nmr}$ ($CDCl_3$) δ (ppm)

20.7 (CH_3-CO-O-), 23.2 (CH_3CO-NH), 48.3 (C_5), 52.6 ($COOCH_3$), 57.8 (C_4), 62.1 (C_9), 67.7, 70.9 (C_7, C_8), 75.9 (C_6), 107.6 (C_3), 145.1 (C_2), 161.5 (C_1), 170.2, 180.3, 170.7, (acetyl -C = O x 4).

Preparation of (4)

Compound (3) (50 mg, 0.11 mmol) was dissolved in anhydrous methanol (5 ml) containing sodium methoxide (8 mg, 0.15 mmol). The mixture was stirred at room temperature for 2 hours and concentrated to dryness *in vacuo*. The residue was taken up in water (3 ml), stirred at room temperature for 1.5 hours, adjusted to pH 6-7 with Dowex 50 x 8 (H^+) resin, and then lyophilised to afford the title compound (4) (35 mg, 94%). i.r. (KBr)cm^{-1} 3400 (br.-OH), 2100 ($-N_3$), 1714 (carbonyl). ^{1}H-nmr (D_2O) δ(ppm). 2.06 (5, 3H, acetyl CH_3), 3.64 (dd, 1H, $J_{9',8}$6.3Hz, $J_{9',9}$11.8Hz, $H_{9'}$), 3.65 (dd, 1H, $J_{7,6}$3.9Hz, $J_{7,8}$6.8Hz, H_7), 3.88 (dd, 1H, $J_{9,8}$2.6Hz,

$J_{9,9'}$ 11.8Hz, H_9), 3.94 (m, 1H, $J_{8,7}$ 6.8Hz, $J_{8,9}$ 2.6Hz, $J_{8,9'}$ 6.3Hz, H_8), 4.21 (dd, 1H, $J_{5,4}$ 10.4Hz, $J_{5,6}$ 8.9Hz, H_5), 4.31 (dd, 1H, $J_{4,3}$ 2.2Hz, $J_{4,5}$ 2.2Hz, $J_{4,5}$ 10.4Hz, H_4), 4.34 (dd, 1H, $J_{6,5}$ 8.9Hz, $J_{6,7}$ 3.9Hz, H_6) 5.82 (d, 1H, $J_{3,4}$ 2.2Hz, H_3).

Example 2 The preparation of Sodium 5-Acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (6)(4-amino-Neu5Ac2en)

Designations of compounds are as in Figure 2.

Preparation of (5)

Into a solution of methyl 5-acetamido-7,8,9-tri-O-acetyl-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (3) (95 mg, 0.208 mmol) in pyridine (6 ml) was bubbled H_2S for 16 hours at room temperature. The solution was then flushed with nitrogen for 15 minutes, and evaporated to remove pyridine under high vacuum. The residue was chromatographed (silica gel, ethyl acetate/isopropanol/water = 5/2/1) to afford a colourless compound (5) (50 mg, 56%).

MS. (FAB) 431 (M^+ +1), 414 (M^+ $-NH_2$), [α] ^{20}D +34.5° (C1, MeOH). i.r. ($CHCl_3$) cm^{-1} 3400 (br. NH_2), 1740 (carbonyl).

^1H-nmr ($CDCl_3$ + CD_3OD) δ (ppm). 1.96, 2.06, 2.07, 2.10 (s, 12H acetyl CH_3 x 4), 3.81 (s, 3H, $-COOCH_3$), 3.92 (brt, 1H, $J_{5,4}$ & $J_{5,6}$ 10Hz, H_5), 4.17 (dd, 1H, $J_{9',8}$ 7.2Hz, $J_{9',9}$ 12.3Hz, $H_{9'}$), 4.22 (br. dd, 2H, $J_{4,5}$ & $J_{6,5}$ 10Hz, $J_{4,3}$ & $J_{6,7}$ 2.1Hz, H_4 & H_6), 4.71 (dd, 1H,

$J_{9,8}$ 2.6Hz, $J_{9,9'}$ 12.3Hz, H_9), 5.31 (m, 1H, $J_{8,7}$ 4.9Hz, $J_{8,9}$ 2.6Hz, $J_{8,9'}$ 7.2Hz, H_8), 5.45 (d, 1H, $J_{7,6}$ 2.1Hz, $J_{7,8}$ 4.9Hz, H_7), 5.97 (d, 1H, $J_{3,4}$ 2.1Hz, H_3).

^{13}C-nmr ($CDCl_3$ + CD_3OD δ (ppm)

20.2, 20.3 (CH_3-CO-O-), 22.3 (CH_3-CO-NH), 48.2(C_5), 50.4(C_4), 52.0 ($COOCH_3$), 62.1(C_9), 67.8, 71.2 (C_7, C_8),

76.5(C_6), 112.5(C_3), 143.6(C_2), 162.0(C_1), 170.2, 170.4, 170.8, 172.2. (acetyl -C = O x 4).

Preparation of (6)

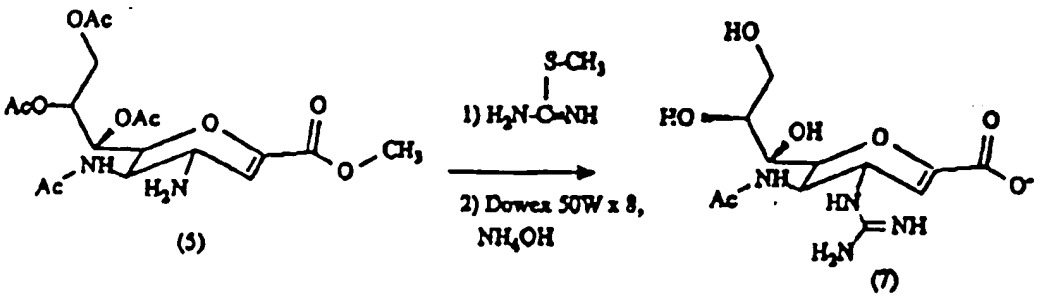
Compound (5) (50 mg, 0.116 mmol) was dissolved in anhydrous methanol (5 ml) containing sodium methoxide (12.4 mg, 0.23 mmol). The mixture was stirred at room temperature for 1.5 hours and evaporated to dryness *in vacuo* at 30° C. The residue was stirred in water (3 ml) at room temperature until TLC (silica gel, ethyl acetate/methanol/0.1 N HCl = 5/4/1) indicated that hydrolysis was complete. The solution (pH about 10.5) was then gradually adjusted to around pH 7.5 by Dowex 50 x 8 (H^+) resin. As soon as the pH of the solution reached 7.5, the suspension was quickly filtered by a press filter. The filtrate was lyophilised to afford the title compound (6) (30 mg, 83%).

^{1}H-nmr (D_2O) δ (ppm). 2.07 (S, 3H, acetyl CH_3), 3.59 - 3.70 m, 2H, H_7 & $H_{9'}$), 3.89 (dd, 1H $J_{9,8}$2.6Hz, -$J_{9,9'}$11.8Hz, H_9), 3.95 (m, 1H, H_8), 3.99 (brd, 1H, $J_{4,5}$10.6Hz, H_4), 4.21 (brt, 1H, $J_{5,4}$ & $J_{5,6}$10.6Hz, H_5), 4.29 (brd, 1H, $J_{6,5}$10.6Hz, H_6), 5.66 (d, 1H $J_{3,4}$1.9Hz, H_3).

Example 3 The preparation of Ammonium 5-Acetamido-4-guanidino-2,3,4,5-tetradeoxy-D *glycero*-D-*galacto*-non-2-enopyranosate

Into a solution of S-methylisourea (546 mg, 3 mmol) in water (15 mL) at ice-bath temperature, methyl-5,7,8,9-tri-O-acetyl-4-amino-2,3,4,5-tetradeoxy-D-*glycero*-D-*galacto*-non-2-enopyranosate (5) (40 mg, 0.093 mmol) was added. The reaction mixture was stirred at 5°C for seven days and poured onto a column of Dowex 50W X 8 (H^+) resin (35 mL). The column was then washed with cold water (700 mL) and eluted with 1.5 M NH_4OH solution. The eluate (120 mL) was concentrated to dryness under high vacuum. The resulting residue was chromatographed (silica gel; solvent system 1: ethyl acetate/isopropanol/water, 1/5/1; solvent system 2: 75% isopropanol) to provide the title compound (7) (8 mg, 24.5%).

The overall reaction scheme is shown below:



Compound (7) gave a strong, positive Sakaguchi reaction, indicating the presence of a guanidine group. NMR data for compound (7) are given below. ^{1}H-nmr (D_2O + CD_3OD) δ (ppm).

2.06(s, 2H, acetyl CH_3), 3.60(br. d., 1H, $J_{7,8}$ 9.4 Hz, H_7), 3.63(dd, 1H, $J_{9',8}$ 6.2 Hz, $J_{9',9}$ 11.8 Hz, $H_{9'}$), 3.76(br. d., 1H, $J_{4,5}$ 9.4Hz, H_4), 3.87(dd, 1H, $J_{9,8}$ 2.6 Hz, $J_{9,9'}$ 11.8 Hz, H_9), 3.93(ddd, 1H, $J_{8,7}$ 9.4 Hz, $J_{8,9}$ 2.6 Hz, $J_{8,9'}$ 6.2 Hz, H_8), 4.01(dd, 1H, $J_{5,4}$ 9.4 Hz, $J_{5,6}$ 10.6 Hz, H_5), 4.20(br. d., 1H, $J_{6,5}$ 10.6 Hz, H_6), 5.63 (d, 1H, $J_{3,4}$ 2.1 Hz, H_3).

Example 4 Inhibition of influenza virus neuraminidase

An *in vitro* bioassay of the above-described compounds against N2 influenza virus neuraminidase was conducted, following Warner and O'Brien (1979), Warner and O'Brien, Biochemistry, 1979 18 2783-2787. For comparison, with the same assay the K_1 for 2-deoxy-N-acetyl-α-D-neuraminic acid, was determined to be 3×10^{-4} M.

Values for K_i were measured via a spectrofluorometric technique which uses the fluorogenic substrate 4-methylumbelliferyl N-acetylneuraminic acid (MUN), as described by Meyers et al., Anal. Biochem. 1980 101 166-174. For both enzymes, the assay mixture contained test compound at several concentrations between 0 and 2 mM, and approximately 1 mU enzyme in buffer (32.5 mM MES, 4 mM $CaCl_2$, pH 6.5 for N2; 32.5mM acetate, 4 mM $CaCl_2$, pH 5.5 for *V. cholerae* neuraminidase).

The reaction was started by the addition of MUN to final concentrations of 75 or 40 μM. After 5 minutes at 37° C, 2.4 ml 0.1 M glycine-NaOH, pH 10.2 was added to 0.1 ml reaction mixture to terminate the reaction. Fluorescence was read at

excitation 365 nm, emission 450 nm, and appropriate MUN blanks (containing no enzyme) were subtracted from readings. The K_i was estimated by Dixon plots (1/fluorescence versus compound concentration). Results are summarized in Table 1.

Table 1

Inhibition of influenza virus neuraminidase in vitro

Compounds	K_i (M)
2-deoxy-N-acetyl-α-D-neuraminic acid	3×10^{-4}
sodium 2,3-dideoxy-α-D-galacto-2-octulosonate	1×10^{-3}
sodium 2,3,5-trideoxy-5-acetamido-α-D-galacto-2-octulosonate	5×10^{-5}
2,3-dideoxy-α-D-glycero-D-galacto-2-nonulosonic acid	2×10^{-2}
2-α-fluoro-N-acetylneuraminic acid	4×10^{-5}
sodium N-acetyl-2-deoxy-2α-allyl-thioneuraminate	1×10^{-5}
sodium 5-acetamido-4-azido-2,3,4,5-tetra-deoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate	2×10^{-6}
sodium 5-acetamido-4-amino-2,3,4,5-tetra-deoxy-D-*glycero*-D-*galacto*-non-2-enopyrano-sonate	6×10^{-8}
	1.9×10^{-7} (N9 neuraminidase)
	1×10^{-8} (N2 virus)
ammonium 5-acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-*glycero*-D-*galacto*-non-2-enopyranosate	1.7×10^{-8}
	$> 5 \times 10^{-8}$ (N9 neuraminidase)
	4.5×10^{-4} (*V. cholerae* neuraminidase; pH 5.8)
	$> 10^{-2}$ (sheep neuraminidase; pH 4.5)

Example 5 *In vivo* anti-viral activity

The compound DANA (2-deoxy-N-acetyl-α-D-neuraminic acid), which was shown in Example 4 to have anti-neuraminidase activity *in vitro*, was tested for anti-viral activity in a standard *in vivo* assay. When administered intranasally to mice before and during challenge with influenza A virus, this compound reduced the titre of virus in lung tissue 1 to 3 days after infection.

Mice were infected intranasally with 50 μl of 10^3 $TCID_{50}$ units/mouse of H2N2 influenza A virus (A/Sing/1/57). The compound was administered intranasally at a dose rate of either 25 mg/kg body weight or 100 mg/kg body weight (50 μl of aqueous solution/mouse) as follows: 24 hours and 3 hours before infection; 3 hours after infection; then twice daily on each of days 1, 2 and 3 after infection.

The mice were sacrificed on days 1, 2 and 3 after infection, their lungs removed and virus titres in the lungs measured. The titres were plotted graphically and expressed as the areas under the curves (AUC). Results are summarized below.

Table 2

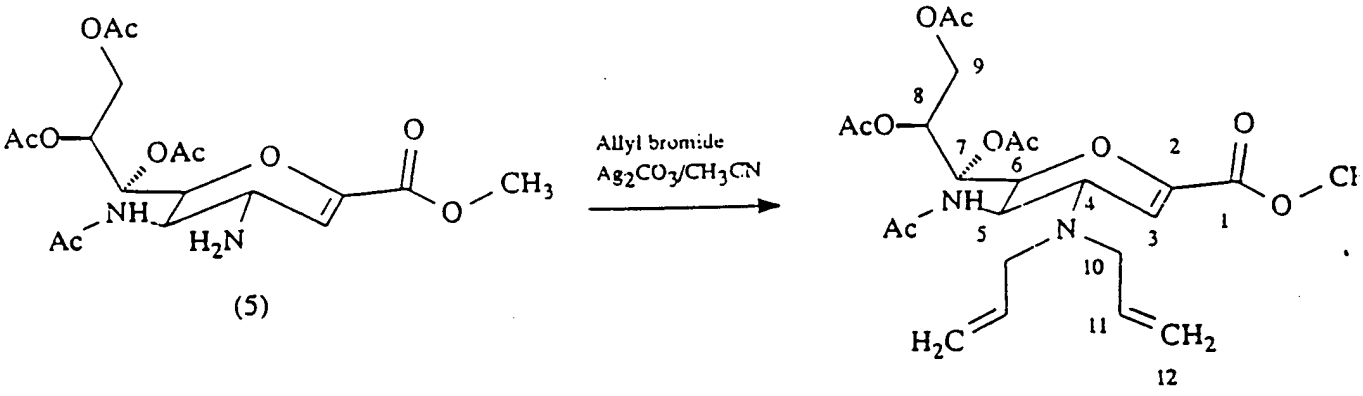
Dose of compound (mg/kg body weight)	Virus titre (AUC) compared to untreated infected mice
25	57%
100	19%

The 4-amino compound of Example 2 showed similar potency to DANA when given intranasally to mice at a single dose level of 25 mg/kg body weight.

In light of the fact that FANA was hitherto thought to be inactive *in vivo*, see Palese & Schulman, *op. cit.*, the high antiviral activity revealed when DANA was administered intranasally to mice is especially surprising. It appears that the route of administration may be signficant in this regard, since DANA is rapidly excreted when given by other routes. See Nöhle et al., Eur. J. Biochem. 1982 126 543-48.

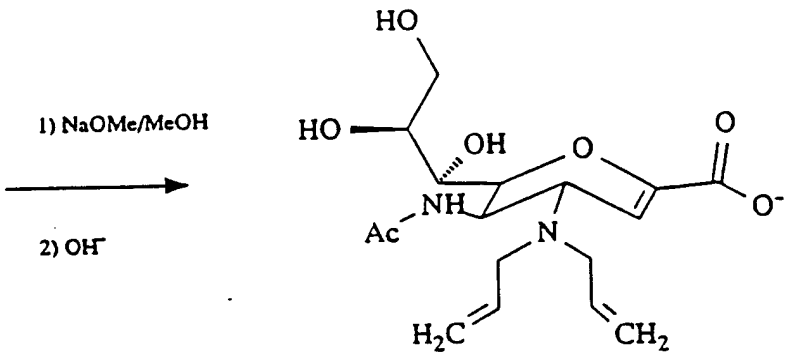
Example 6

Sodium 5-Acetamido-4-*N*,*N*-diallylamino-2,3,4,5-tetra-deoxy-D-glycero-D-galacto-non-2-enopyranosonate. (9).



(5)

(8)



(9)

Into a solution of allyl bromide (60mg, 0.5mmol) and methyl 5-acetamido-7,8,9-tri-*O*-acetyl-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-enopyranosonate (5) (90mg, 0.209mmol) in acetonitrile (5mL), was added silver carbonate (116mg, 0.418mmol). The mixture was stirred and protected from light at room temperature for 16 h. The resulting suspension was filtered, and the filtrate was evaporated to dryness. The residue was subjected to flash-column chromotography (silica gel, ethyl acetate containing 10% methanol) to afford compound (8) (85mg, 80%).

^{1}H-nmr ($CDCl_3$) δ(ppm) 1.94, 2.05, 2.06, 2.11(s, 12H, acetyl CH_3 x4), 2.97(dd, 2H, $J_{10a,10b}$& $J_{10'a,10'b}$-14.3Hz, $J_{10a,11}$& $J_{10'a,11'}$7.6Hz, H_{10a}& $H_{10'a}$), 3.24(dd, 2H, $J_{10b,10a}$& $J_{10'b,10'a}$14.3Hz, $J_{10b,11}$& $J_{10'b,11'}$4.9Hz, H_{10b}& $H_{10'b}$), 3.58(dd, 1H, $J_{4,3}$2.4Hz, $J_{4,5}$9.3Hz, H_4), 3.79(s, 3H, $COOCH_3$), 4.12-4.26(m, 3H, H_6, $H_{9'}$, H_5), 4.70(dd, 1H, $J_{9,8}$2.6Hz, $J_{9,9'}$-12.3Hz, H_9), 5.09(dd, 2H, $J_{12cis,11}$& $J_{12'cis,11'}$10.6Hz, J_{12gem}& $J_{12'gem}$≈1.5Hz, H_{12cis}& $H_{12'cis}$), 5.14(dd, 2H, $J_{12trans,11}$& $J_{12'trans,11'}$17.7Hz, J_{12gem}& $J_{12'gem}$≈1.5Hz, $H_{12trans}$& $H_{12'trans}$), 5.27-5.32(m, 2H, H_8 & -CONH-), 5.55(dd, 1H, $J_{7,6}$2.1Hz, $J_{7,8}$4.7Hz, H_7), 5.72(m, 2H, H_{11}& $H_{11'}$), 6.07(d, 1H, $J_{3,4}$2.4Hz, H_3).

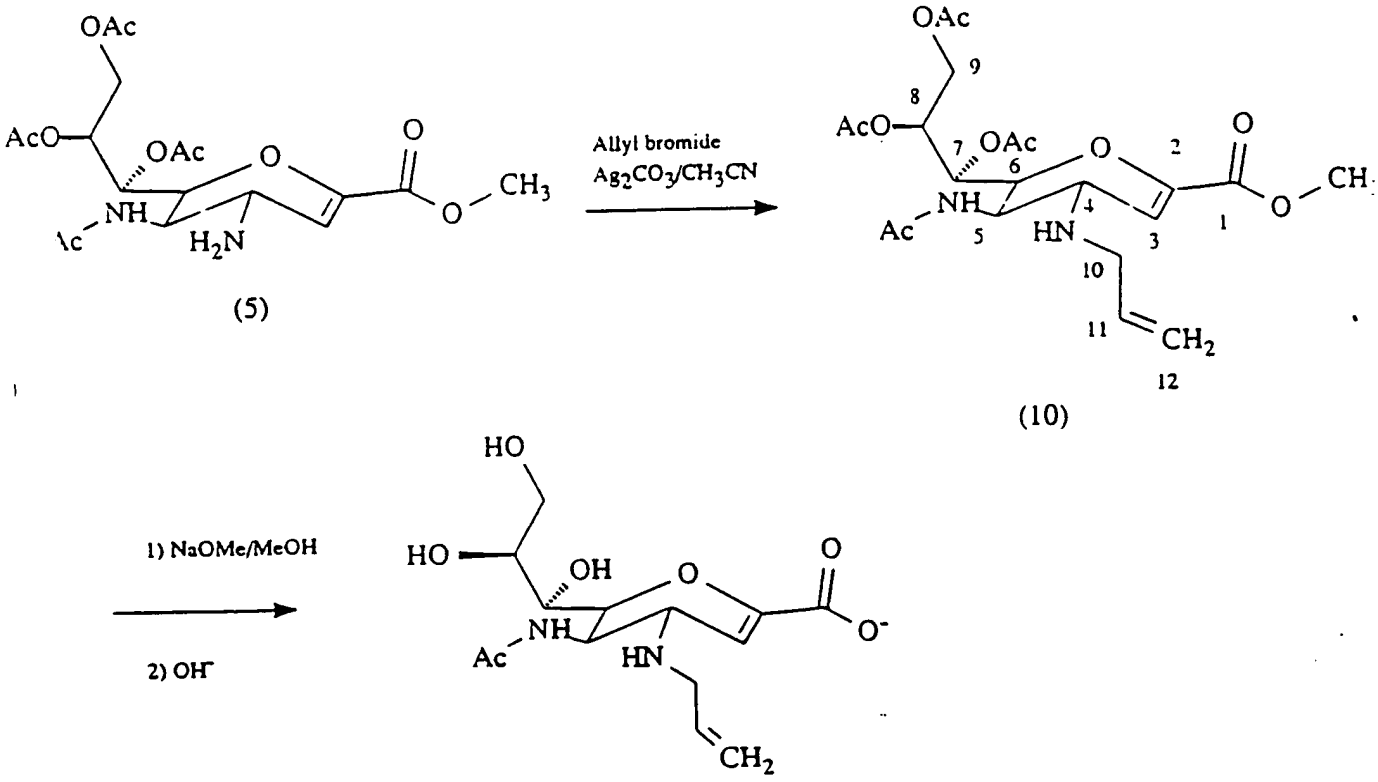
Compound (8) (80mg, 0.156mmol) was dissolved in anhydrous methanol (10mL) containing sodium methoxide (16.2mg, 0.30mmol). The solution was stirred at room temperature for 2 h, then evaporated to dryness. The residue was taken up in water (5mL), and left at room temperature for 2 h. The resulting solution was neutralized with Dowex 50 x 8 (H^+) and freeze-dried to afford compound (9) (49mg, 80%).

^{1}H-nmr (D_2O) δ(ppm) 1.94(s, 3H, Acetyl CH_3), 3.24-3.44(m, 4H, H_{10} x2 & $H_{10'}$ x2), 3.48-4.33(m, 7H, H_4, H_5, H_6, H_7, H_8, H_9 & $H_{9'}$), 5.24-5.29(m, 4H, H_{12} x2 & $H_{12'}$ x2), 5.69(d, 1H, $J_{3,4}$≈2Hz, H_3), 5.73-5.76(m, 2H, H_{11} & $H_{11'}$)

Compound (9) was found to be a competitive inhibitor against influenza virus neuraminidase with a Ki value of 4 x 10^{-6}M.

Example 7.

Sodium 5-Acetamido-4-*N*-allylamino-2,3,4,5-tetra-deoxy-*D*-glycero-*D*-galacto-non-2-enopyranosonate (*11*).



To a solution of allyl bromide (48mg, 0.40mmol) and compound (5) (155mg, 0.36mmol) in acetonitrile (5mL) was added silver carbonate (107mg, 0.38mmol). The mixture was stirred, whilst protected from light, at room temperature for 16 h. The resulting suspension was filtered off, and the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column (ethyl acetate / isopropanol / water = 5:2:1). Fractions with an Rf value of 0.5 were combined and evaporated to dryness to afford compound (10) (53mg, 32%). The starting material (5) with an Rf value of 0.3 (61mg, 39%) and *N*, *N*-diallyl derivative (8) with an Rf value of 0.9 (20mg, 11%) were recovered respectively.

^{1}H-nmr ($CDCl_3$) of compound (10) is shown as follows (δppm) 1.96, 2.05, 2.06, 2.11(s,

12H, Acetyl CH_3 x4), 3.25(dd, 1H, $J_{10a,10b}$-14.1Hz, $J_{10a,11}$5.8Hz, H_{10a}), 3.37(dd, 1H, $J_{10b,10a}$-14.1Hz, $J_{10b,11}$5.9Hz, H_{10b}), 3.43(dd, 1H, $J_{4,3}$3.1Hz, $J_{4,5}$7.5Hz, H_4), 3.79(s, 3H, $COOCH_3$), 4.09(ddd, 1H, $J_{5,4}$7.5Hz, $J_{5,NH}$9.1Hz, $J_{5,6}$8.1Hz, H_5), 4.21(dd, 1H, $J_{9',8}$7.1Hz, $J_{9',9}$-12.2Hz, $H_{9'}$), 4.30(dd, 1H, $J_{6,5}$8.1Hz, $J_{6,7}$4.1Hz, H_6), 4.63(dd, 1H, $J_{9,8}$3.2Hz, $J_{9,9'}$-12.2Hz, H_9), 5.09(dd, 1H, $J_{12cis,11}$10.2Hz, $J_{12cis,12trans}$-1.3Hz, H_{12cis}), 5.18(dd, 1H, $J_{12trans,11}$17.1Hz, $J_{12trans,12cis}$-1.3Hz, $H_{12trans}$), 5.36(ddd, 1H, $J_{8,7}$4.2Hz, $J_{8,9}$3.2Hz, $J_{8,9'}$7.1Hz, H_8), 5.57(dd, 1H, $J_{7,6}$4.1Hz, $J_{7,8}$4.2Hz, H_7), 5.65(d, 1H, $J_{NH,5}$9.1Hz, -CONH-), 5.83(dddd, 1H, $J_{11,12trans}$17.1Hz, $J_{11,12cis}$10.2Hz, $J_{11,10a}$5.8Hz, $J_{11,10b}$5.9Hz, H_{11}), 6.09(d, 1H, $J_{3,4}$3.1Hz, H_3).

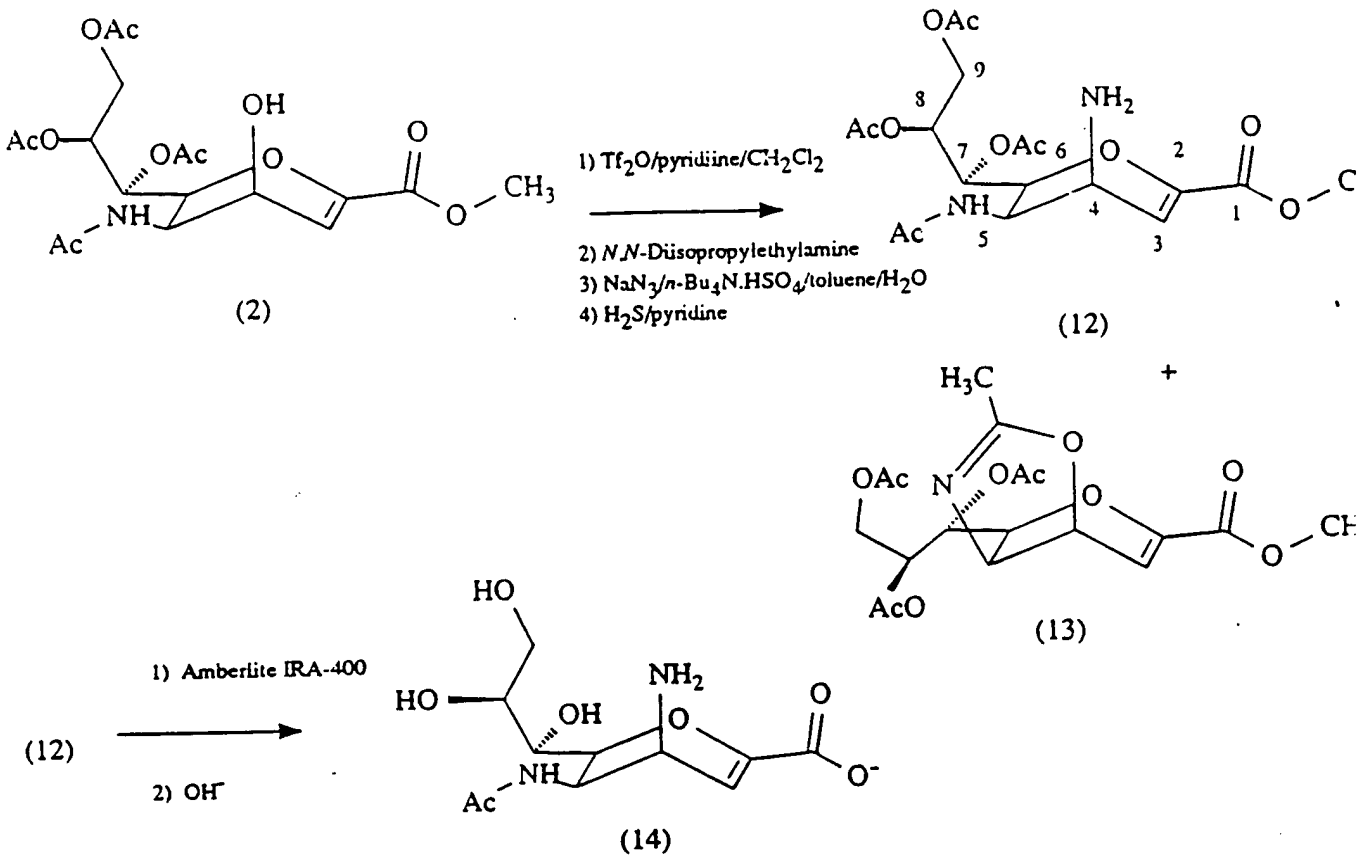
Compound (10) (50mg, 0.11mmol) was stirred in anhydrous methanol (5mL) containing sodium methoxide (12mg, 0.225mmol) at room temperature for 2 h, then evaporated to dryness. The residue was redissolved in water (5mL) and allowed to stand at room temperature for 2 h before being neutralized with Dowex 50x8 (H^+) resin. The aqueous solution was freeze-dried to afford compound (11) (31mg, 78%).

^{1}H-nmr (D_2O) δ(ppm) 2.02(s, 3H, CH_3CO), 3.42(dd, 1H, $J_{10a,10b}$-13.4Hz, $J_{10a,11}$6.6Hz, H_{10a}), 3.52(dd, 1H, $J_{10b,10a}$-13.4Hz, $J_{10b,11}$6.3Hz, H_{10b}), 3.51-4.27(m, 7H, H_4, H_5, H_6, H_7, H_8, H_9 & $H_{9'}$), 5.30(dd, 1H, $J_{12cis,12trans}$≈1.5Hz, $J_{12cis,11}$10.3Hz, H_{12cis}), 5.34(dd, 1H, $J_{12trans,12cis}$≈1.5Hz, $J_{12trans,11}$17.7Hz, $H_{12trans}$), 5.72(d, 1H, $J_{3,4}$2.4Hz, H_3), 5.89(dddd, $J_{11,10a}$6.6Hz, $J_{11,10b}$6.3Hz, $J_{11,12cis}$10.3Hz, $J_{11,12trans}$17.7Hz, H_{11}).

Compound (11) is a competitive influenza virus inhibitor of neuraminidase (N2 & N9) with a Ki value of 2.5 x 10^{-6}M.

Example 8

Sodium 5-Acetamido-4-amino-2,3,4,5-tetra-deoxy-D-glycero-D-talo-non-2-enopyranosonate (14).



To a stirring solution of compound (2) (500mg, 1.04mmol) in anhydrous dichloromethane (8mL) containing pyridine (205mg, 2.6mmol) at -30°, was added dropwise a solution of trifluoromethanesulphonic anhydride (Tf_2O) (367mg, 1.3mmol) in dichloromethane (2mL) over a period of 20 minutes. The reaction mixture was then stirred at -30° for 5 h, and finally evaporated to dryness under reduced pressure. The resulting residue was stirred in dry DMF containing *N,N*-diisopropylethylamine (194mg, 1.5mmol) at room temperature for 16 h. The reaction mixture was concentrated under high vacuum to remove DMF. The residue was then stirred in a two-phase mixture of toluene (5mL) and water (5mL) containing tetra-*n*-butylammonium hydrogen sulphate (950mg, 2.8mmol) and sodium azide (137mg,

2.1mmol). The mixture was stirred at room temperature for 16 h and then evaporated to dryness. The residue was partioned between ethyl acetate (50mL) and water (15mL), with the organic layer washed successively with water (5mL x 2), and then evaporated to dryness. The residue was taken up in pyridine (5mL), bubbled with H_2S, and then evaporated to dryness. The residue was subjected to flash-column chromotography (silica gel, the first solvent system was ethyl acetate, the second solvent system was ethyl acetate / iso-propanol / H_2O : 5/2/1). The ethyl acetate eluate contained compound (13) (260mg, 53%). The fractions with a positive ninhydrin reaction, collected from the second solvent system, were combined and evaporated to dryness to afford compound (12) (32mg, 6.5%).

MS (FAB), 431 ($M^+ + 1$), 414 ($M^+ - NH_2$).

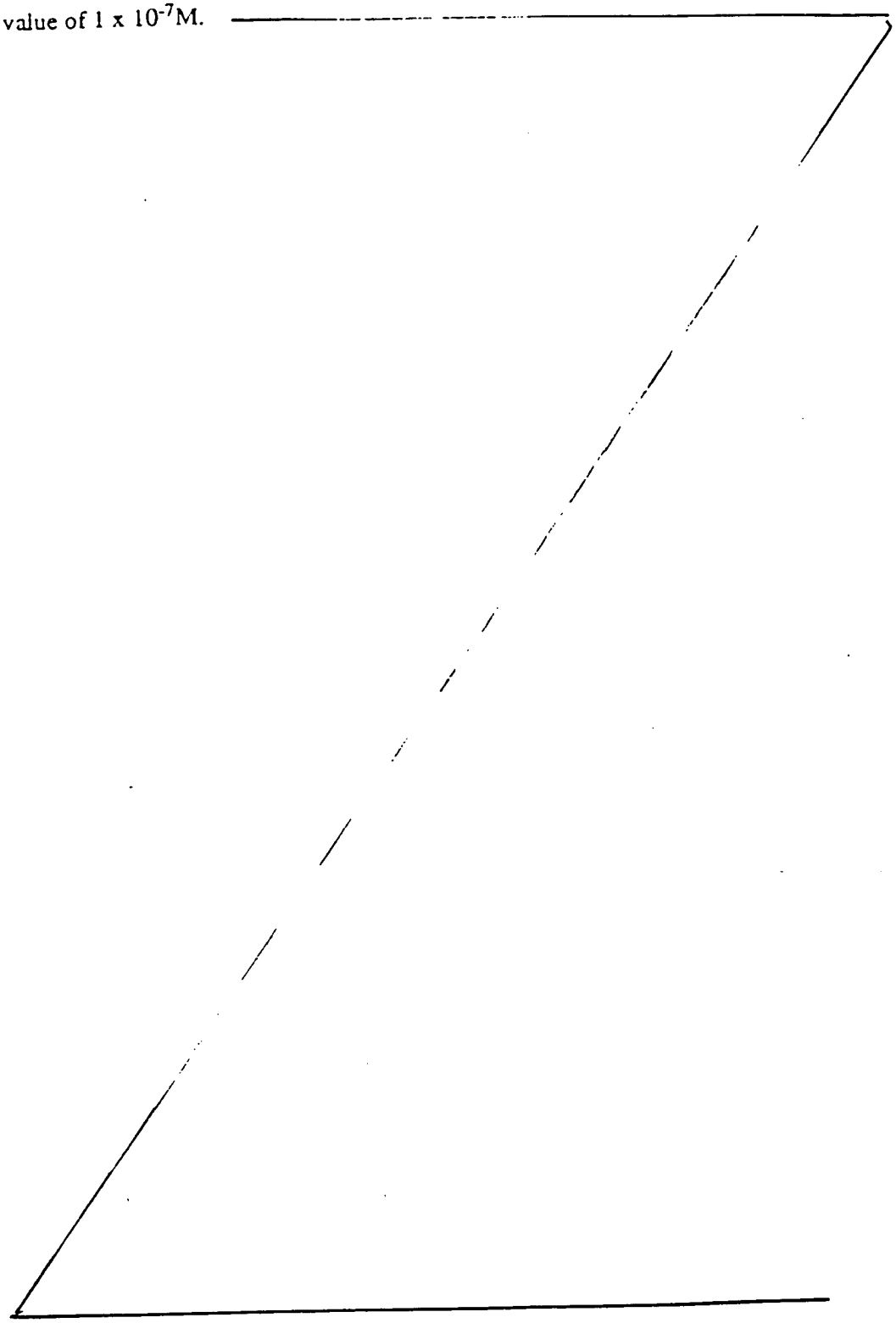
^{1}H-nmr ($CDCl_3 + CD_3OD$) δ(ppm) 1.96, 2.06, 2.08, 2.09(s, 12H, Acetyl CH_3 x4), 3.52(dd, 1H, $J_{4,3}$5.5Hz, $J_{4,5}$4.5Hz, H_4), 3.80(s, 3H, $COOCH_3$), 4.16(dd, 1H, $J_{6,5}$10.2Hz, $J_{6,7}$2.3Hz, H_6), 4.17(dd, 1H, $J_{9',9}$-12.4Hz, $J_{9',8}$7.3Hz, $H_{9'}$), 4.23(dd, 1H, $J_{5,6}$10.2Hz, $J_{5,4}$4.5Hz, H_5), 4.73(dd, 1H, $J_{9,9'}$-12.4Hz, $J_{9,8}$2.7Hz, H_9), 5.34(ddd, 1H, $J_{8,7}$4.7Hz, $J_{8,9}$2.7Hz, $J_{8,9'}$7.3Hz, H_8), 5.45(dd, 1H, $J_{7,6}$2.3Hz, $J_{7,8}$4.7Hz, H_7), 6.12(d, 1H, $J_{3,4}$5.5Hz, H_3).

^{13}C-nmr ($CDCl_3 + CD_3OD$) δ(ppm) 20.7($\underline{C}H_3C(O)O$-), 23.1($\underline{C}H_3C(O)N$-), 43.8(C_5), 46.2(C_4), 52.4($COO\underline{C}H_3$), 62.3(C_9), 68.3, 71.8(C_7, C_8), 73.0(C_6), 111.5(C_3), 143.8(C_2), 162.4(C_1), 170.3 & 170.8($CH_3\underline{C}O$ x4).

Compound (12) was stirred in anhydrous methanol (5mL) containing Amberlite IRA-400 (OH^-) resin (100mg) at room temperature for 3 h. Following filtration, the filtrate was evaporated to dryness. The residue was dissolved in water (5mL) and adjusted to pH13 with 0.1M NaOH. The aqueous solution was stirred at room temperature for 2 hr and then neutralized with Dowex 50 x 8 (H^+) resin. After filtration, the filtrate was lyophilized to afford compound (14) (16mg, 70%), which was positive in the ninhydrin reaction.

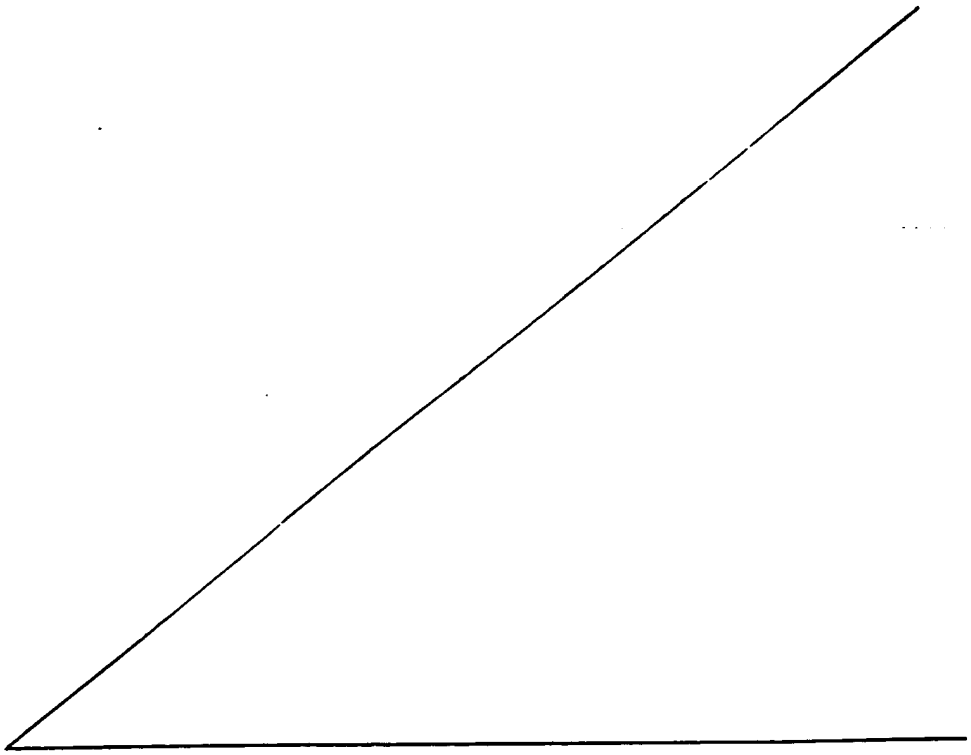
^{1}H-nmr (D_2O) δ(ppm) 2.10(s, 3H, CH_3CO), 3.67-3.76(m, 2H, H_4 & $H_{9'}$), 3.92(dd, 1H, $J_{9,8}$2.8Hz, $J_{9,9'}$-11.9Hz, H_9), 3.90-4.02(m, 2H, H_7 & H_8), 4.37-4.44(m, 2H, H_5 & H_6), 5.81(d, 1H, $J_{3,4}$5.14Hz, H_3).

Compound (14) was found to be a strong inhibitor of N2 & N9 influenza virus neuraminidase with a Ki value of 1×10^{-7}M.



Pharmaceutical Compositions

A pharmaceutical formulation within the present invention combines, with an active agent that binds the viral neuraminidase active site and displays *in vivo* anti-viral activity, a carrier for the active agent which is pharmaceutically acceptable. A pharmaceutically acceptable carrier is a solid, liquid or gaseous material that can be used as a vehicle for administering a medicament because the material is inert or otherwise medically acceptable, as well as compatible with the active agent, in a particular context of administration. In addition to a suitable excipient, a pharmaceutically acceptable carrier can contain conventional additives like diluents, adjuvants, antioxidants, dispersing agents and emulsifiers, anti-foaming agents, flavour



correctants, preservatives, solubilizing agents and colourants.

The nature of the excipient used with an anti-viral agent, pursuant to the present invention, is largely a function of the chosen route of administration, as discussed, for example, in REMINGTON'S PHARMACEUTICAL SCIENCES (E.W. Martin ed.) and in PHARMACEUTICAL DOSAGE FORMS AND THEIR USE (H. Hess ed.) Hans Huber Publ., 1985, the respective contents of which are hereby incorporated by reference. Preferably, the pharmaceutical compositions of the present invention are provided in a unitary-dosage form which is suitable for administration intranasally, orally, buccally or sublingually.

In accordance with the present invention, a pharmaceutical composition is advantageously delivered to the throat, nasal cavity or lungs, the intranasal route of administration being especially preferred. Delivery of an active agent to the nasal cavity can be achieved with preparations of the present invention that take the form, for example, of an aerosol or vapour, a nasal spray or nose drops, or an inhalation powder. For these applications, it may be appropriate for the active agent to be micronized, for example, to a particle size on the order of 5 microns or less.

Suitable means for effecting delivery by direct application to the mucosal lining or via inhalation are well known to the art, for example, in the context of treating asthma. In this category

are squeeze-bottle devices (nebulisers) and pressurized packs, for delivering a solution of the active agent as a spray into the nose, and conventional insufflators like the Spinhaler turbo-inhaler and liquid aerosol "puffers" (Spinhaler is a registered trade mark of Fisons Corporation), which deliver metered doses of a pharmaceutical preparation.

If the active agent is delivered from solution, as would typically be the case for a nasal spray or nose drops, the carrier preferably comprises distilled water that is both sterile and substantially free of fever-inducing (pyrogenic) substances, thereby to minimise the incidence of medical complications relating to contamination. Suitable propellants to comprise carriers for use in administration by pressurized aerosol are well known, including halogenated fluorocarbon gases, carbon dioxide, and nitrogen. See, e.g., Lachman et al. *in* THE THEORY AND PRACTICE OF INDUSTRIAL PHARMACY (Lea and Febiger, Philadelphia), 1976. In addition, a carrier for administration via intranasal delivery or insufflation may contain oleic acid or some other pharmaceutically acceptable stabiliser, as well as a surface-active agent, e.g., a detergent like Tween 80 or Span 80, in order to enhance uptake of the active agent.

Conventional forms which are favoured for oral administration include lozenges and pastilles, sublingual and buccal tablets, and oral sprays. Numerous carriers suitable for these forms are

known, including solid pulverulent carriers comprising a simple sugar or corresponding alcohol (lactose, saccharose, sorbitol, mannitol, etc), a starch such as potato starch, corn starch or amylopectin, cyclodextrin, a cellulose derivative, and gelatine. Liquid carriers can also be employed to form suspensions, syrups, elixirs and solutions containing the active agent. Non-aqueous vehicles which are suitable as liquid carriers in this regard include almond oil and other edible oils, fractionated coconut oil, oily esters, propylene glycol and ethyl alcohol.

In formulating a pharmaceutical preparation of the present invention for oral administration, a solid carrier would typically be mixed with a lubricant, such as magnesium stearate, calcium stearate or a polyethylene glycol wax, and then compressed into tablet form. In keeping with common practice, tablets can be coated with a concentrated sugar solution which may contain components like gum arabic, gelatine, talcum and titanium dioxide. Alternatively, tablets can be coated with a lacquer dissolved in a readily volatile organic solvent.

A pharmaceutical composition within the present invention contains a virus-inhibiting amount of an active agent as described above. The optimum dosage of the active compound will vary with the particular case, and can be determined routinely in the clinical context, which may be prophylactic or therapeutic. 'Prophylactic' treatment is to be understood to mean treatment intended to prevent or

retard second-cycle infection as defined below, thus preventing the establishment of the complete clinical manifestations of the disease caused by that virus. 'Therapeutic' treatment is to be understood to mean treatment intended to alleviate the symptoms and severity of infection which is already established, by disrupting release of virus particles and thus preventing further cycles of viral replication. Generally, the amount of active agent present in a pharmaceutical composition of the present invention should be sufficient to inhibit at least second-cycle infection by orthomyxovirus or paramyxovirus in an animal. That is, an initial viral infection of a cell culminates in the assembly and budding of virus particles at the cell-membrane surface, which would be followed in the normal course by release of the particles and infection thereby ("second-cycle infection") of other cells. A suitable amount of active agent to include in a pharmaceutical composition of the present invention would thus retard at least this second cycle of infection by virus, it is thought by inhibiting the action of neuraminidase that results in release of virus particles from the membrane surface.

For administration by inhalation, the daily dosage as employed for treatment, according to the present invention, of an adult human of approximately 70 kg body weight will range from 1mg to 1000 mg, preferably between 5 mg and 500 mg, and may take the form of single or multiple doses, e.g., one to six times a day. For oral administration,

the daily dosage (again, for treatment of a 70 kg adult) will typically range from about 1 mg to 5 g, preferably between 5 mg and 2 g, and may be given, for example, in single to four doses per day. It will therefore be convenient for a pharmaceutical composition of the present invention to contain active (antiviral) agent at a concentration in the range of 0.000001 to 100 mg/ml.

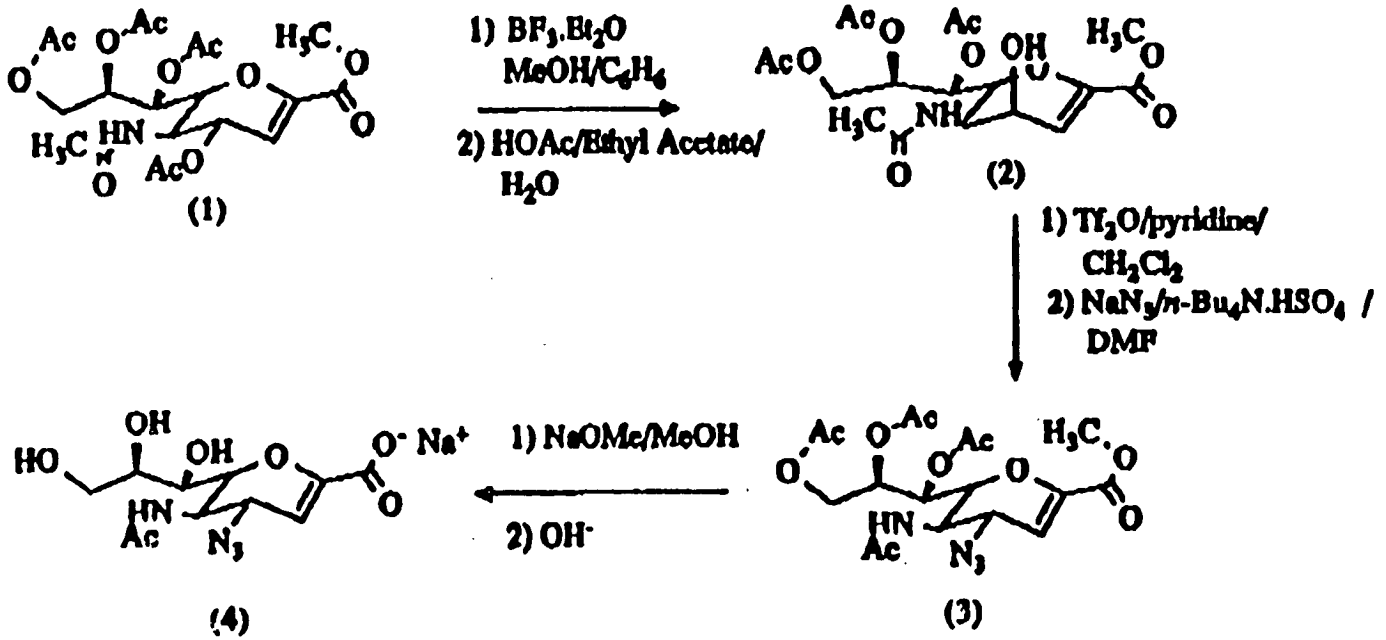
Other objects, features and advantages of the present invention will become apparent from the preceding detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DATED this 19th day of October 1990

BIOTA SCIENTIFIC MANAGEMENT PTY LTD
By Their Patent Attorneys

GRIFFITH HACK & CO
Fellows Institute of Patent
Attorneys of Australia

FIGURE 1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(2)
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CH_2Cl_2
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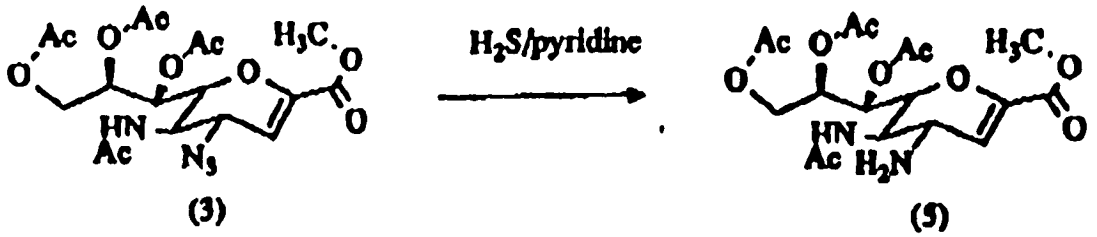
DMF
1) NaOMe/MeOH
2) OH^-
(3)
(4)

FIGURE 2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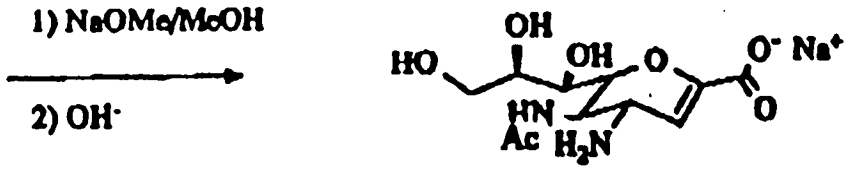
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HO
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O
O- Na+
HN
Ac
H2N
O

(6)

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International publication No.	WO91/16320 PCT/AU91/00161
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